The Development of Alcyonium digitatum, with some notes on the Early Colony Formation.

By

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With Plates 3–5 and 51 Text-figures.

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1. Preface.

I take this opportunity of thanking the Council of the Marine Biological Association for the use of a table while working out these results. Also I gladly acknowledge the kindly and continued help given me by Dr. Allen and the various members of his staff. This generous aid rendered

the work very much easier, and enabled me to get all the necessary stages with a minimum amount of labour. My warmest thanks are also due to Prof. Hickson, of the Manchester University, for many useful hints and for reading through and criticising the completed paper.

2. Introduction to the Development of Alcyonium Digitatum.

The broad outlines of the development of Alcyonium digitatum were worked out by A. Kowalevsky in 1873 (**8**), and amplified later by Hickson (**2**, **3**, **4**, and **4a**), and it has been the object of this paper to add further details to the information given by these authors. On the whole the results agree, except in some details concerning the sequence of development of certain organs.

There is an interesting general resemblance between the accompanying sketches of the segmenting egg, the planula and the early fixed polyp, and those previously given by:

(1) de Lacaze-Duthiers, for Astroides calycularis (**10**).

(2) Wilson, for Renilla and Leptogorgia (**16**).

(3) Kowalevsky and Marion, for Sympodium and Clavellina (**9**).

A comparison of the plates given by these authors with those at the end of the present paper will demonstrate this. In particular, Pl. xiii, fig. 6, of de Lacaze-Duthiers' memoir (**10**) would illustrate excellently the way in which Alcyonium larvæ settled in the finger-bowls in which they were reared, during the experiments now described. Therefore, A. digitatum bears out the collected evidence that the Anthozoa develop roughly according to one and the same plan.

3. Methods used to Preserve and Stain the Alcyonium Material.

(1) Preserving fluids.

(*a*) Schaudinn's fluid (corrosive sublimate and absolute alcohol).

(*b*) Corrosive acetic.

(*c*) Bouin's picro-formol-acetic.

The above three reagents appeared equally good for preserving all stages, except when the structure of the spicules was required. Perhaps (*c*) was the best general preserving fluid.

(*d*) Osmic acid, for preparations showing spicules and nematocysts.

(2) Staining reagents.

(*a*) Delafield's hæmatoxylin. For morulæ, and well-stained segmentation spindles; also for gland cells in the œsophagus and ventral mesenteric filaments.

(*b*) Ehrlich's hæmatoxylin, as (*a*).

(*c*) Borax-carmine and picro-nigrosin. For planulæ and all subsequent stages; for structure of mesoglœa.

(*d*) Ranvier's picro-carmine, followed by Kernschwarz, after fixing with osmic acid. For spicule structure. The picro-carmine stains the nuclei, while the Kernschwarz stains the spicule and its surrounding protoplasm.

(*e*) Iron-brazilin. Good for all settled stages, gland cells, etc., in combination with some plasma stain, e. g. safranin.

4. General Account.

Ripe male and female colonies[1] of Alcyonium digitatum are brought in by the trawlers in the Plymouth district from early December to early February, and fertilised eggs were obtained:

(1) Between January 27th and February 13th, 1912.

(2) Between December 10th and February 10th, 1912–1913.

(3) Between December 14th and February 10th, 1913–1914.

[1] Hermaphrodite colonies occasionally occur, male and female polyps being present. Hermaphrodite individuals are also sometimes found in these colonies, such exceptions finding a parallel in the case of Corallium nobile (11).

These eggs were successfully reared in the Plymouth laboratory. The above data shows that A. digitatum spawns during two of the coldest and stormiest months of the year, when the supply of material is necessarily uncertain, and therefore the work of collecting the various segmentation stages is, as a rule, unavoidably spread over the whole of the spawning season. The colonies used for this paper came from the trawling ground between the Dodman and the Eddystone, i.e. outside and some miles west of Plymouth Sound. They reached the laboratory in good condition in buckets of sea-water, and after being well washed the ripest colonies were selected, the choice being easily made, as ripe ova are deep reddish-yellow in colour, and ripe sperm sacs a very opaque white. The colonies were then placed by themselves in one of the laboratory tanks, through which a constant stream of sea-water circulated, care being taken to avoid overcrowding. As the colonies generally remain healthy for only a few days in the laboratory tanks, even under the most favourable conditions, rarely expanding fully and possibly never feeding, those chosen must be in the best condition and ready to spawn.

Some hours later the circulation was stopped and the female colonies soon spawned in the still water. It seems essential to have the water still, as this is favourable to the necessary expansion of the very sensitive polyps. Simultaneously the male polyps discharged from their mouths large quantities of spermatozoa which swam freely and fertilised the floating ova. On microscopic examination the latter were then seen to be completely covered by a delicate fringe of spermatozoa which gave them a pseudo-ciliate appearance, but the "cilia," i. e. the tails, of the spermatozoa were non-motile.

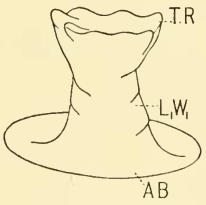
It was impossible to distinguish the particular sperm which fertilised the ovum, or to say when this occurred. Different colonies began to extrude their ova at varying times of the day or night (unlike Renilla, which spawns only between 6 and 7 a.m. (16)).

The eggs of any one colony were passed out con-

tinuously for several days, until the spawning was complete.

On January 3rd, 1913, a spawning colony was placed in a beaker of water and closely watched from 11 a.m. to 3 p.m. The majority of the polyps were expanded while their tentacles were half retracted; others were only partly expanded (c.f. Text-fig. 1). Five eggs were successively extruded at regular intervals from the mouth of one polyp during a period of fifteen minutes. They passed up the stomodæum one by one and after escaping from the mouth remained in contact with the tentacles and oral surface until some slight

TEXT-FIG. 1.



Solitary polyp, lateral view. Tentacles retracted, body slightly contracted. This also illustrates the appearance of the colonial polyp while spawning.

motion of the water finally dislodged them, when they floated upwards. In some cases several eggs were seen in the stomodæum simultaneously, one below the other, and in squeezing upwards through this narrow tube they became temporarily oval but regained their round shape after extrusion. The transparent membrane which surrounded them before spawning, and which always envelops eggs taken forcibly from the mesenteries, was thrown off during the process of spawning, and the empty membranes were ejected into the water after the ova.

Although artificially fertilised ova did in some cases segment satisfactorily, it was found more practicable for rearing on a large scale to collect the fertilised ova from the tank where they were naturally spawned and fertilised. To do this the water was siphoned over into white dishes, from which the eggs were removed with a pipette.

The eggs are opaque, very yolky, and reddish-yellow in colour. They are about 0·5 mm. in diameter, and float at

TEXT-FIGS. 1A–8.

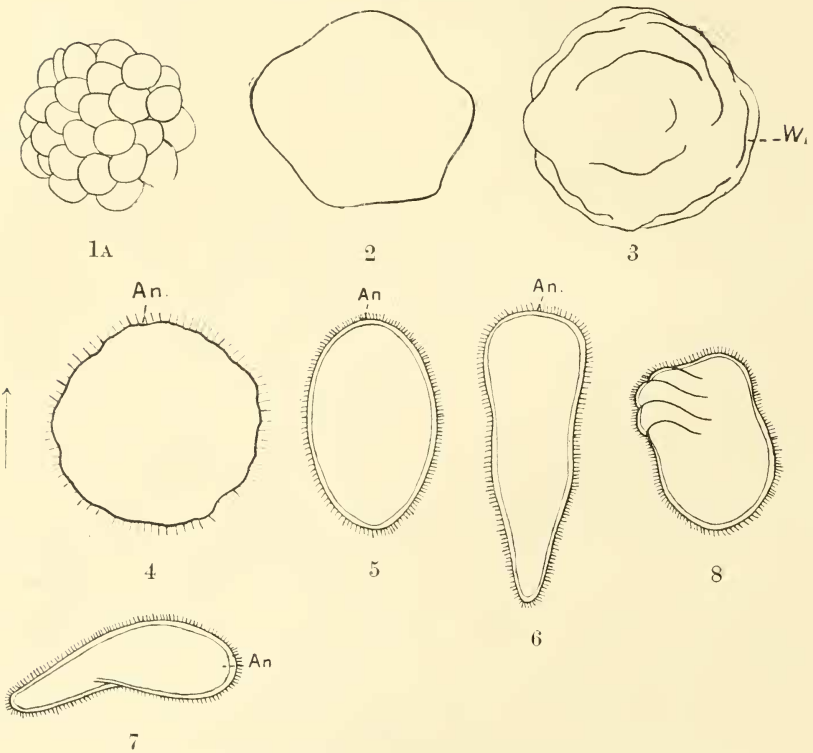


Fig. 1A. Sixty-four celled stage, × 46. Fig. 2. Rather late pre-planula, with prominent lobes, × 56. Fig. 3. Latest stage pre-planula; lobes softened into shallow wrinkles; seventy-two hours old, × 62. Fig. 4. Round ciliate planula of sinuous outline, succeeding Fig. 3. The arrow indicates the direction of progress, × 52. Fig. 5. Oval planula of smooth outline, twenty-four hours older than last figure, × 40. Figs. 6 and 7 show planarian-like movements of long thin planula, × 40. Fig. 8. Planula showing lateral ridges when contracted due to irritation, × 40.

various depths in the tank as though their specific gravity is very near that of sea-water. After segmentation has begun

they increase slightly in size, and become somewhat paler in colour, and are thus distinct from newly-spawned eggs to the naked eye.

They were pipetted into finger-bowls of "outside" water (water brought into the laboratory from outside Plymouth Sound, and therefore in especially good condition), and development took place at the ordinary and by no means constant temperature of the laboratory. The developing eggs appeared to do equally well in outside, Berkefeld-filtered or ordinary tank water, and many larvæ went through all the stages of development, settled and produced tentacles in the laboratory tank where the eggs were spawned. Twenty-four hours after spawning the embryos were all morulæ, more or less advanced.

Fresh colonies were continually added to the tank, and spent and unhealthy ones removed during the whole of the spawning season. Judging by the proportion of ripe ones brought in, this season reaches its height towards the end of January, and soon declines after that date.

The segmenting egg (Text-fig. 1A) is in all stages typically spherical, though during segmentation some examples may become temporarily oval, regaining their globular shape later. At the close of the morula stage the embryo undergoes a curious change in shape. About the twenty to twenty-fourth hour the whole surface of the sphere is slowly drawn out into irregular blunt prominences with corresponding depressions (Text-fig. 2), the component cells meantime undergoing a modification of structure and the segmentation cavity gradually disappearing. This condition lasts for some time, but about the forty-fourth hour the knobs begin to withdraw again. Wilson (16) mentions a similar stage for Renilla and Leptogorgia, but apparently did not investigate it closely. As this definite stage is followed by the swimming planula, it was convenient to call it the "pre-planula" stage. By the end of the third day of development the pre-planula no longer shows definite protuberances and depressions, these having softened down into a gently wrinkled outline (Text-fig. 3).

On the fourth day the cells near the centre of the solid preplanula begin to disintegrate, and so the larva again becomes hollow and passes on into a free-swimming planula stage with a definitely marked anterior pole. This planula developes cilia, is at first roughly spherical and of sinuous outline (Text-fig. 4), but lengthens somewhat in a few hours into a highly contractile oval planula still of wavy outline. By the fifth day it is a smooth oval planula swimming rather slowly at various levels in the water, usually in a horizontal plane (Text-fig. 5). Very soon the anterior end broadens and a pear-shaped planula results, which rotates continuously on its long axis while progressing in the water (Text-fig. 6). The reddish-yellow colour of the ovum is still present, but gradually becomes paler as the yolk is absorbed, and the planula increases in length. The larva continually changes its shape, so that measurements of the ever varying length and breadth are rendered difficult. While swimming it exhibits characteristic planarian-like contractile movements, which are represented in Text-figs. 6 and 7, and any irritation causes strong contraction and lateral wrinkling (Text-fig. 8).[1] By the seventh day the planula is very long and slender, measuring 1·3 mm. long and 0·3 mm. wide, but is not very often fully extended. The anterior and aboral pole is deeper in colour than the narrower posterior and oral pole where more yolk has been absorbed (Pl. 3, fig. 1). The surface at this time is abundantly supplied with nematocysts and mucous cells (Pl. 3, fig. 2), the latter being especially numerous at the anterior pole. At first the planulæ swim at varying levels in the bowls, but towards the third free-swimming day they become more sluggish, and most of them keep in a vertical position with the thin aboral end hanging downwards (c. f. de Lacaze Duthiers (**10**), Pl. xiii, fig. 6). Many then sink to the bottom of the dishes in this position (possibly this is an

[1] All the young stages are very sensitive to heat, and the microscope lamp has to be used with caution while examining them or they quickly die.

attempt to settle), but usually they get caught up here in their own mucus and eventually degenerate.

Some larvæ develop more quickly than others, but usually on the fourth free-swimming day, i. e. the seventh day of development, many larvæ settle. After hovering motionless for some time with the broad anterior pole apparently touching the chosen place for settling, the planula becomes attached (Text-fig. 9). In this they agree with Sympodium and Clavellina (**9**). A thin disc of opaque white mucus (the mucous plug) fastens them to the substratum (Pl. 3, fig. 3, *M. P.*), this mucus being secreted by the mucous cells in the ectoderm of the anterior end. In bowls containing water only, the circulation set up by the constantly varying temperature of the laboratory carried many larvæ to the top of the water so that on becoming sluggish they were caught up in the surface film and settled there, either on the film itself or on the glass wall of the dish (c f. de Lacaze-Duthiers (**10**), Pl. xiii, fig. 6). In the former case the settled polyps also developed perfectly, hanging upside down from the film until this was disturbed, sending them down to the bottom.

In a certain number of bowls, wherein small Pecten shells were placed, the larvæ settled in great numbers on both surfaces of the shells and on the parts of the dish sheltered by them, i. e. the base and lower part. In these cases the circulation is modified by the presence of the shells, so the larvæ are less numerous in the surface film. In some dishes the planulæ did not settle until the fifth, sixth, to fourteenth day of free-swimming life, and although these are results obtained under laboratory conditions, a varying length of free-swimming life would obviously help dispersal in the sea, and give more larvæ a chance to find Ascidians, Chætopterus tubes, Hydroids, Pecten shells or other suitable objects to settle on. The planulæ often settled so close to one another as to render a group difficult to distinguish with the naked eye from a young colony. All the larvæ did not settle, and

TEXT-FIGS. 9–14.

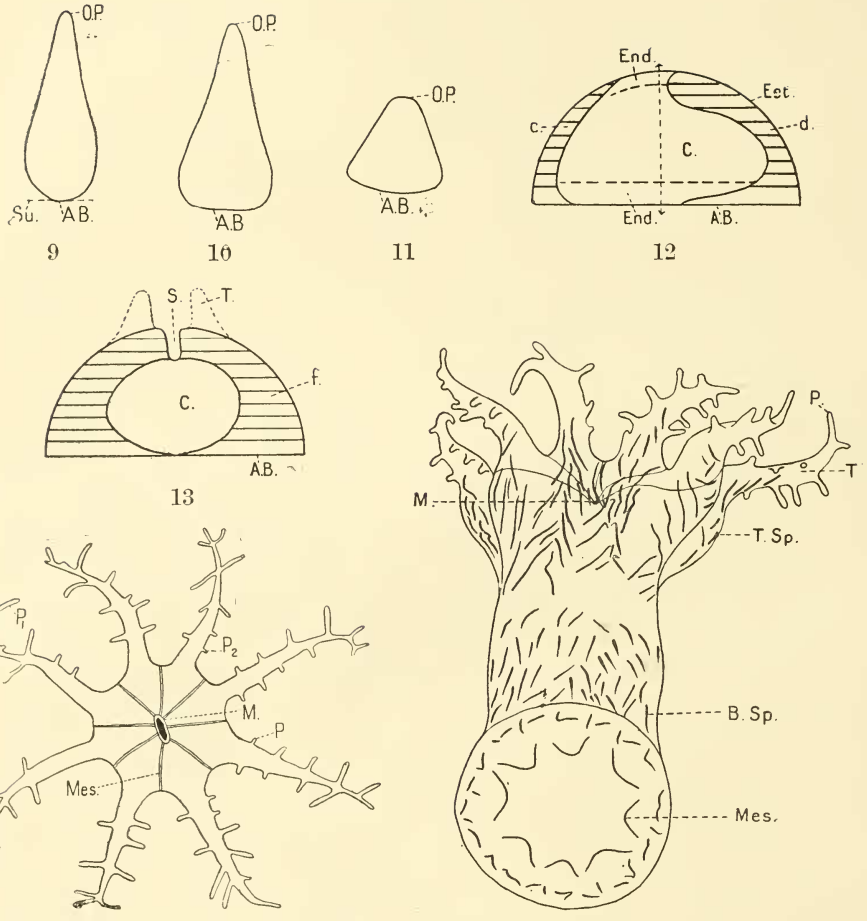


Fig. 9. Planula just settled (cilia not represented). Fig. 10. Same planula a little later, length shortening; still ciliate. Fig. 11. Same, length considerably shortened, cilia retracted. Fig. 12. Vertical section of young polyp arranged to illustrate two stages in the growth of the mesentery. The vertical dotted line divides the two. The mesentery (*c*) is intermediate in development between mesentery (b_1) of Text-fig. 40, and mesentery (*d*) in this figure. A broken line indicates the inner edge of the endoderm. Fig. 13. Similar section on the third day when the stomodæum is developing. The tentacles have arisen, but are shown by dotted lines as they are alternate with the mesenteries. Fig. 14. Lateral view of polyp about eighteen days fixed. Tentacles bearing five to six pinnules and spicules showing well. Fig. 15. Oral view of polyp with five to six pinnules. Tentacles well expanded, mouth almost closed (from bowl, × 25).

those which passed beyond the fifteenth to sixteenth free-swimming day without fixing eventually degenerated.

The base of the newly fixed pear-shaped larva looks like a round pinkish disc from below, the planula for a time retaining its power of planarian-like contraction and expansion (Text-fig. 10). Soon the cilia[1] are retracted (Text-fig. 11), and the fixed polyp shortens first to a stumpy oval shape, and then to a flat mound shape by the end of the first twenty-four hours. At the beginning of the second day the mesenteries show by transmitted light as eight equidistant vertical ridges growing up from the base of the lateral walls (Pl. 3, fig. 4, *P.M.*). These shallow ingrowths almost meet on the aboral and oral surfaces by the end of the second day (Text-fig. 12, *d.*), while eight small blunt conical outgrowths alternating with them on the oral surface indicate the tentacles (Text-fig. 13, and Pl. 3, fig. 5,). On the third day the polyp has increased in size, and the stomodæum is formed by an invagination of the oral surface, within the circle of the tentacles (Pl. 3, fig. 6, *M.*). This tube deepens rapidly, and by degeneration of its base the cœlenteron is put in communication with the exterior on the fourth day. Meanwhile the tentacles and body continue to lengthen (Pl. 3, figs. 6 and 7) and soon the former develop two rows of pinnules, seven being the maximum number developed in the laboratory on any one row (Text-figs. 14 and 15). The polyps had on an average produced in each row:

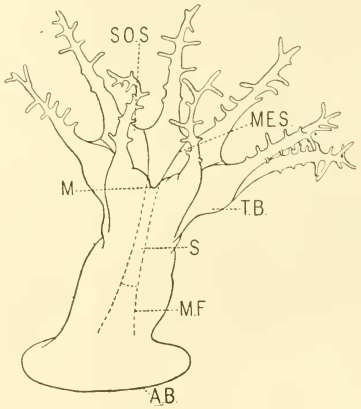
One pinnule by the sixth day.
Two pinnules by the seventh day.
Two to three pinules by the eighth day.
Three to four pinnules by the tenth to twelfth day.
Five pinnules by the twelfth day.
Six pinnules by the eighteenth day.
Seven pinnules by the twenty-first day.

And while the older pinnules were carried along during development towards the tips of the tentacles, the new ones

[1] No cilia were seen on the ectoderm of the solitary polyp in any subsequent stage.

developed in succession below them, so that the youngest pinnule was always at the base (Text-fig. 15, P_1 and P_2). The numbers were variable in the two rows on any one tentacle, and on the eight tentacles of any one polyp. The tentacles were faint pink in colour at first, but this changed to pale cream during development. Some polyps seemed to develop rather quicker than others, as did the planulæ. When fully grown the measurements of the solitary polyp are only about half the corresponding ones of the colonial polyp, i. e. about

TEXT-FIG. 16.



Solitary polyp, settled fourteen to twenty days; tentacles bearing six pinnules, diameter of base 1·2 mm. Lateral view, well expanded (from finger-bowl), × 10.

6 mm. in height, tentacles 3 mm. long, and diameter of base 1·2 mm., while it is much more opaque, and consequently the stomodæum shows less plainly and the mesenteric filaments are obscured. As soon as the tentacles are long enough, they curl gracefully about in the water, seeking food (Text-fig. 16). Any vibration causes them to retract in part or wholly, the polyp in a complete state of retraction appearing as a small pink mound with an opaque centre. The spicules in the tentacles and body nearly meet in the solitary polyp (Text-fig. 14, *T. Sp.*, and *B. Sp.*), whereas they are widely separated in the individuals of older colonies. Some

polyps lived in finger-bowls in the laboratory for about three months, but eventually died without forming colonies. They seemed healthiest when the bowls were immersed in much larger cylinders of sea-water, with the growth of algæ and diatoms checked by limiting the amount of light, and with frequent changes of water. In finger-bowls through which air was constantly bubbled the polyps were nearly always expanded, but in others they were nearly always closed until dusk, although they usually opened if the bowls were removed from their containing cylinders for examination. Hence the polyps seem sensitive to light, currents in the water, and temperature. No regular alternation of the expanded and contracted stages was observed.

5. Colony Formation.

Some of the polyps produced one bud in the laboratory, and one produced two daughter polyps. The finger-bowls were difficult to keep clean, as the algæ and diatoms in the fine townetting, which was added from time to time as food, settled down rapidly as a thick greenish coating. Therefore at this stage, as it seemed unlikely that the colonies would continue to develop in the laboratory, two bowls containing numerous solitary polyps and young colonies were taken out on March 17th, 1914, to Cawsand Bay, just outside Plymouth Sound, to continue their growth. They were fastened in wicker stands which were suspended inside a box-like raft into which the sea penetrated, and were brought in temporarily for examination on the following dates, after which they were always returned:

(1) April 30th, after one and a half months on the raft.
(2) July 15th, after four months on the raft.
(3) September 16th, after six months on the raft.

This experiment was entirely successful, and the polyps gave rise to disc-shaped colonies in the early stages of which the new individuals arose according to a definite plan. As the polyps increased in number the parent individuals seemed

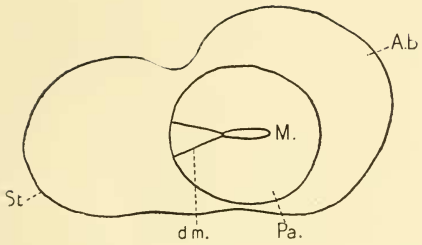
to grow larger themselves, and the number of tentacular pinnules increased gradually from seven to twelve or thirteen. When last examined (September 16th, 1914), although the best developed colony consisted of about thirty-two polyps, they still all grew out of a common flat encrusting base. The following account of the production of new polyps from the parent individual by stolonal gemmation is a summary of observations made on the young stages in the laboratory and the older ones from the raft. Young colonies trawled from the Rame–Eddystone grounds, and growing on Chætopterus tubes, Polycarpa, etc., were found quite similar in their plan of growth.

Detailed Account of Colony Formation.

About three weeks after fixation the circular base of the polyp produced a blunt outgrowth opposite the two dorsal mesenteries (Text-fig. 17, *St.*). This stolon increased in size, became of circular outline and separated from the parent polyp by a slight constriction. Soon it produced a bud which rapidly grew into a second polyp (Text-figs. 18 and 19), with its dorsal mesenteries adjacent to the dorsal mesenteries of the parent.[1] Then a second bud formed quite similarly from

[1] Hickson (2) describes an Alcyonium polyp which bore one bud. The figure he gives (Pl. iii, fig. 24), indicates budding from the lateral wall of the parent polyp, and not stolonal gemmation. It is difficult to reconcile this example with the present account, and no explanation can be offered.

Fig. 17. Aboral view of polyp, showing outgrowth of stolon previous to formation of first bud. Fig. 18. Similar view of well-expanded polyp with one bud. The two pairs of dorsal mesenteries are seen opposite one another, and the long axes of the mouths lie along one line. Fig. 19. Lateral view of colony of two. Size of polyps very similar (from finger-bowl). Fig. 20. Oral view of parent polyp and two buds (from Chætopterus tubes). [In Figs. 20 and 22–25 the common stolon is ruled in with faint lines.] Fig. 21. Aboral view of polyp with three buds (raft). Figs. 22–24. Oral view of colonies showing four, six and eight buds respectively. Buds numbered in order of appearance. Fig. 25. Colony with three rows of buds—aboral view (raft).



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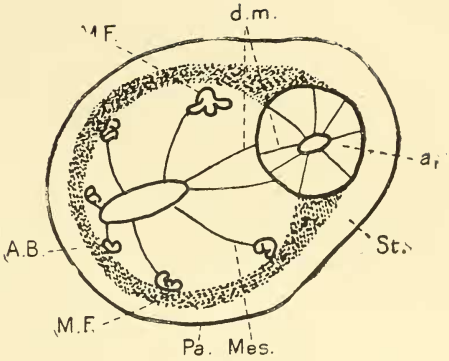

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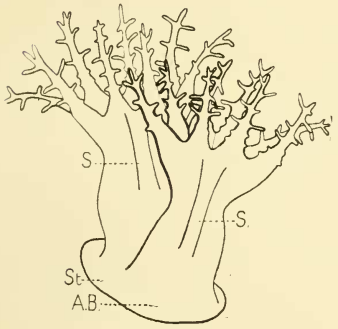

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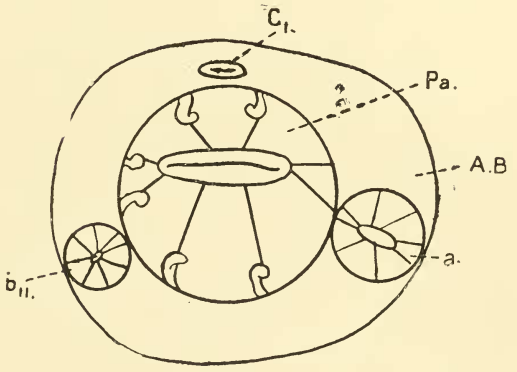

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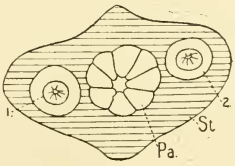

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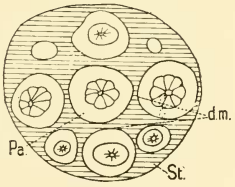

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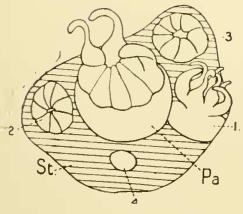

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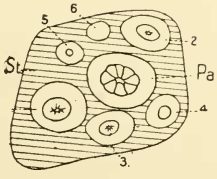

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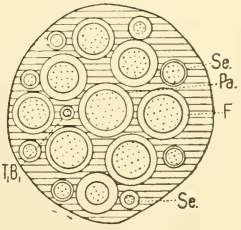

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a stolon-like outgrowth on the opposite side of the parent's base and with its dorsal mesenteries likewise turned towards the original polyp (Text-fig. 20). By April 30th the parent polyp bore ten pinnules on the tentacles, many parents showing two buds, and one bearing three (Text-fig. 21). The second bud showed no definite relation to any mesentery, while the third one lay between the first and second (Text-fig. 21, a_1, b_{11}, and c_1). By mid-July the colony, which consisted on April 30th of four individuals, had altogether produced eight buds by stolonal gemmation, and these lay in a circle around, and all with their dorsal mesenteries turned toward the original polyp (Text-fig. 22), and therefore with the long axis of the mouth lying always along a radius. Possibly this arrangement would facilitate a current of water and food upwards through the young buds from the parent polyp, as the dorsal filaments produce an upward current in Alcyonium (Hickson, **3a**, and Wilson, **17**). Other polyps in the bowl had also produced eight buds, and the rest from six buds downwards (Text-figs. 23 and 24). On September 16th it was found that several colonies had produced about thirty-two individuals, while others were not nearly so far advanced. The younger colonies still retained their circular outline, but the older ones had lost it. One had become quite oval because barnacles had prevented its lateral expansion.

After the production of the first circle of eight buds a second row forms outside these and alternate with them, giving two concentric rows (Text-fig. 25). The second row then increases greatly in number, and, later, young buds appear between the parent of the colony and the first row (Text-fig. 25). In still older colonies this regular system becomes obscured, young and old polyps being irregularly scattered throughout the colony. It was found that colonies dredged in the Sound at this date and on the Rame–Eddystone grounds contained a fair proportion of examples of similar size to those reared on the raft.

6. Food.

This appears to be almost wholly animal. In only one instance was evidence found of any vegetable matter being ingested—when a desmid was seen embedded in an endoderm cell in one of the ventral mesenteric filaments. The polyps reached quite an advanced stage of development while simply using up the embryonic yolk. Several pinnules had developed before this was exhausted, in bowls to which no food had been added. Cultures of Nitzschia, Pleurococcus, and other very small green algæ were tried as food with no success. Very fine plankton was added regularly to some bowls and to the water of the rearing tank, as adult colonies are known to thrive on Nauplii and small Copepods (**12**), and on this food the polyps developed six to seven pinnules, and small colonies were produced. The reddish remains of a fairly large copepod was one day found in a polyp, and on two other occasions Temora longicornis was swallowed, while Balanus nauplii were also accepted. However, those polyps kept in Cawsand Bay flourished best, and while in the laboratory for examination were frequently seen catching and swallowing the larvæ of Leptoclinum, which was also growing on the dishes. They were successfully fed with these larvæ and with similar larvæ of Botrylloides, from a pipette, and would also take adult individuals removed from these colonies when they were offered. The larvæ were swallowed head first, and the red Botrylloides larvæ could be traced excellently. Stages in the swallowing and disintegration of food exactly like those figured by Miss Pratt (**12**) were obtained. The young colonies showed no evidence of being preyed upon on the raft, nor did the settled polyps in the laboratory tanks, although shrimps ate the eggs and swimming larvæ readily. It was interesting to find a parasitic copepod in the cœlenteron of the polyps of the female colonies feeding on the eggs.

7. Segmentation.

The newly fertilised egg is of about 0·5 mm. diameter, opaque, and full of reddish-yellow yolk. It has accordingly to be rolled round in a suitable vessel of sea-water in order that the lobes and segments may be seen. Hence observations are less easy than in the case of the transparent eggs of Echinus. The eccentric oosperm nucleus is somewhat oval in shape, and resembles that figured by Hill (**5**). The problem of the maturation and fertilisation of the egg together with the first divisions of the oosperm nucleus was not attempted, as it is rather outside the scope of this work.

The outstanding feature of the early segmentation in A. digitatum is its great irregularity of procedure. It would seem as though the many ways of reaching the morula stage were highly variable and unimportant, the resulting embryos, however, being apparently of one kind. This bears out Wilson's remarks on Renilla (**16**), and is the first of a series of similarities shown during the development of these two Alcyonaria. No polar bodies are extruded and no outward sign marks the time of actual fertilisation. Before any permanent alteration in appearance occurs the ovum seems to make violent but unsuccessful attempts at division, i. e. the spherical egg becomes temporarily polygonal, the surface being drawn out into irregular high ridges with corresponding depressions, possibly witnesses of internal activity (Text-fig. 26). These ridges disappear after some time and the egg returns to its spherical condition. The whole process may be repeated with no apparent result. In one case the ridges softened down into eight vaguely defined, lighter coloured areas which covered the whole surface of the egg, but these eventually disappeared, leaving a spherical egg as before. In other cases the ovum became temporarily long and oval, but regained its globular shape later. In artificial fertilisations the time elapsing between the adding of sperm to the bowl of ova and the above-described attempts at division varied from half an hour to two and a half hours in different cases. The

interval between the end of this stage and the subsequent formation of definite lobes, and again between the protrusion of lobes and the actual production of blastomeres was also very variable. Before segmentation begins the surface of the egg usually becomes pushed out into eight equal or unequal lobes (Text-fig. 27). However, sixteen lobes are sometimes protruded instead of eight (Text-fig. 28). In one such case the first lobe slowly formed about two hours after fertilisation, and soon afterwards the second, third, and fourth lobes followed, all at one pole (Text-fig. 29, *l.*). Next larger lobes slowly formed towards the opposite pole (Text-fig. 29, *L.*), and while these increased in number all the lobes became more prominent. Two and a half hours after fertilisation the lobes were still increasing in number, and half an hour later still sixteen were counted embracing the whole surface of the egg. Sections of this stage revealed one nucleus only, but in sections of other similar embryos the nucleus was rapidly dividing into daughter nuclei. When only eight lobes form they are necessarily larger in size than when sixteen are protruded (cf. Text-figs. 27 and 28). Great variability, however, exists in the protrusion of lobes; many eggs seem to throw them out in a very irregular manner, the lobes themselves varying in number: thirty-two, sixty-four, and other numbers have been counted.

In the majority of ova, while eight equal or unequal superficial lobes are protruded, the centre yolk remains at first undivided (Pl. 3, fig. 8, *C. M.*). Subsequently the grooves between the lobes deepen, extending towards the centre of the egg, and almost cutting it into eight segments. Finally the segments become rounded off from one another at the centre, leaving a little granular protoplasmic waste in the newly formed segmentation cavity (Text-fig. 30, and Pl. 3, fig. 9, *S. C.*). This cavity persists until the end of the morula stage. Usually the oosperm nucleus is found lying near the region where the first lobes form, and, though it may divide up into eight daughter nuclei while the lobes are being protruded, the fission of the oosperm nucleus is sometimes

delayed until the lobing is complete. Sections of ova during the continual subdivision of the parent nucleus show beautiful

TEXT-FIGS. 26–34.

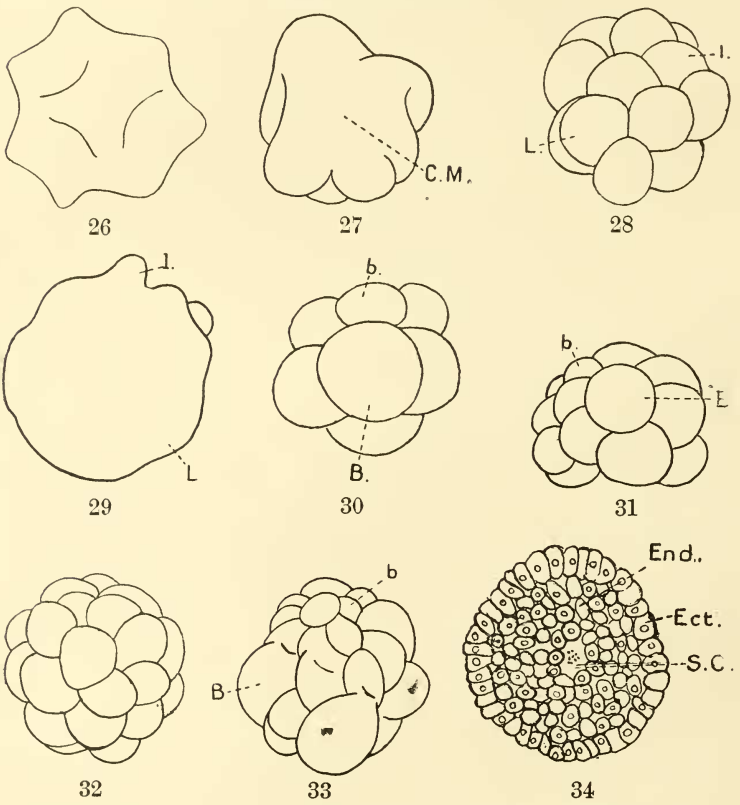


Fig. 26. Egg making abortive attempts to divide, × 32. Fig. 27. Egg with eight unequal lobes, the primary nucleus and central protoplasmic mass being still undivided, × 48. Fig. 28. Sixteen lobes protruded, eight large and eight small, × 50. Fig. 29. Early lobed stage, two hours after fertilisation, × 54. Fig. 30. Egg segmented into four large and four small cells, × 42. Fig. 31. Sixteen cells—eight large and eight small, × 42. Fig. 32. Thirty-two celled stage, × 42. Fig. 33. Segmenting egg with many small cells at one pole, and fewer large ones at the other, × 45. Fig. 34. Sagittal section of late morula, just passing into pre-planula stage, × 78.

karyokinetic figures, one daughter nucleus passing to each of the eight lobes (Pl. 3, fig. 10). After the first segmentation the blastomeres may be uniform or may fall into groups of four large and four small cells (Text-fig. 30), or again may be quite irregular in size. The nucleus in each segment now halves, exhibiting meanwhile well-formed spindles, some with equatorial chromosomes and others with the halved chromosomes at each pole. Then the eight cells divide into sixteen, generally eight smaller at one pole and eight larger at the other. All the blastomeres do not divide simultaneously, and hence stages are found with ten, twelve, or fourteen cells only, but an examination of the nuclei shows that the undivided cells will also shortly segment, giving the typical sixteen cell stage. In some fourteen-celled embryos the nuclei of many of the segments had again halved ready for the thirty-two celled stage before the two slowest of the original eight cells had completed their first division. When sixteen lobes are protruded the egg divides immediately into sixteen cells instead of eight (Pl. 3, fig. 11, and Text-fig. 31). These segments again may be equal or unequal, and are, as in the case of the eight cell stage, separated by a segmentation cavity containing a little waste protoplasm. In all cases the sixteen cells halve, giving thirty-two blastomeres, and here again some segments divide very slowly, and so twenty, twenty-four, and twenty-eight celled embryos occur. The thirty-two blastomeres (Text-fig. 32) may or may not be uniform, and the irregularity in all the previously mentioned stages prepares one for and helps to explain other embryos where the segmentation seems quite irregular, or where numerous very small cells form a cap at one pole over a few large cells at the other (Text-fig. 33). Possibly the large amount of yolk in the ovum causes the unequal segmentation as well as the initial futile attempts at division and the retardation of fission in some blastomeres. The thirty-two celled embryo divides repeatedly, giving sixty-four, one hundred and twenty-eight, etc., cells. By this time the embryo has become two-layered, the segments at each division

becoming smaller and more numerous. Between the periods of division the segments are flattened, but just after segmentation they are very prominent, so that the contour alters considerably. The late morula is approximately spherical, and though during late segmentation it may become temporarily oval it soon regains its round shape. The first delamination cleavage occurs when the nuclei of the sixteen cell stage divide (Pl. 3, fig. 11, *D. N.*). Some of the spindles lie along a radius of the sphere, and hence the resulting daughter nuclei lie similarly, so that a cell is split off towards the centre of the embryo in all such cases, and thus an inner endodermic layer arises (Pl. 3, fig. 12, *End.*). Other spindles lie in a plane at right angles to the radius, and hence these daughter cells lie side by side with the parent cell in the outer ectodermic row (Pl. 3, fig. 12, *N.*). It is thus evident that all of the sixteen cells do not simultaneously contribute to the endoderm layer, and while both ectoderm and endoderm cells continue to divide, later radial spindles in the ectoderm afford evidence that the early endoderm cells are continually reinforced from the ectoderm (Pl. 3, fig. 12).[1] Hence from the thirty-two celled stage onwards the larva is two-layered (Text-fig. 34). The outer layer is somewhat irregular at first (Pl. 3, fig. 12), and, as Wilson says of Renilla (**16**), the ectoderm cells dovetail into those forming the inner mass. The endodermic yolk globules are much larger than those of the ectoderm at this stage (Pl. 3, fig. 21). As the number of cells increases the ectoderm becomes a more regular row of cuboid cells, staining much more deeply than the inner layer of larger polygonal endoderm cells (Pl. 3, fig. 21). Towards the end of the morula stage about thirty-two small cells can be counted round the circumference of the sphere, the ectoderm being now columnar (Text-fig. 34). The yolk in the endoderm has been partly used up, the vesicles having become smaller and similar to those in the ectoderm (Pl. 3, fig. 20).

[1] The writer hopes to discuss the question of the origin of the endoderm in greater detail in a subsequent paper.

Early Embryos in Section.

The granular protoplasm of the unsegmented egg is full of yolk globules. These are largest towards the periphery, while a shallow surface layer of the egg is finely granular and devoid of yolk. These areas are still visible in the eight and sixteen cell stages (Pl. 3, fig. 13), the finely granular surface layer being confined to the outer edge of the blastomeres. The yolk distribution in the later morula stages has been already described, the large round nuclei being surrounded by a deeply staining finely granular area (Pl. 3, fig. 20).

Other Types of Segmentation.

One embryo was sectioned in which an irregular outer layer of blastomeres had been cut off from an inner undivided mass.[1] This example agrees with Kowalevsky's description of the segmentation of the egg of Alcyonium (**8**). In this he relates how a complete covering layer of nucleate ectoderm cells of various sizes was cut off from an undivided central yolk mass, which itself split later into a few large cells, while further divisions of all the cells resulted in a morula similar to those now described. Hickson (**4**) records a four-celled embryo, and during the present investigations one embryo was followed to the late morula stage from two unequal blastomeres.

8. The Pre-planula.

After the twentieth hour the spherical morula becomes very gradually distorted, slowly protruding blunt lobes separated by corresponding depressions (Text-fig. 2). It is found that at this time the numerous yolk globules in the columnar ectoderm and the polygonal endoderm are still small and quite similar in the two layers. About the fiftieth hour, when the prominent lobes very slowly begin to soften down again,

[1] Very possibly this egg was unhealthy; it is quoted because Kowalevsky's account of the early development of the egg of Alcyonium differs so greatly from what is described above.

the yolk globules become fewer but larger in size, and continue this decrease in number and increase in bulk for some little time, apparently by fusion of the smaller globules (Pl. 3, fig. 18, and Pl. 3, fig. 14, *Y. V.*). Meanwhile the segmentation cavity, which has persisted until now (Text-fig. 34), disappears. The columnar ectoderm cells increase greatly in length and number, becoming very slender, while smaller rounded ones appear among them (Pl. 3, fig. 15, *Ect.* and *R. C.*). The outer halves of the columnar cells have become finely granular, the inner portions still containing yolk globules (Pl. 3, fig. 15, *Gr. E.* and *Y. E*), while the round cells each contain about four large yolk globules.[1] The innermost endoderm cells, which are also the largest, now begin to degenerate (Pl. 3, fig. 16, c.), and by the continued absorption of these cells a series of cavities forms which presently fuse into one—the cœlenteron—the wall of which consists of a layer of endoderm about three cells deep, with the columnar ectoderm outside. As development continues the endodermic layer becomes progressively thinner by the degeneration and ultimate absorption of the inner cells, until it consists of only one row of columnar cells about half as long and twice as broad as the columnar ectoderm cells. Round cells are at this time found lying between the tall ectoderm cells at both their inner and outer ends (Pl. 4, fig. 27, a drawing of the next subsequent stage, which would also serve as a figure of this stage). At times the columnar ectoderm cells proliferate very rapidly, and so the ectoderm becomes temporarily multilayered, soon, however, regaining its normal columnar character (Text-fig. 35). In the final stages (Text-fig. 3), the ectoderm cells of the pre-planula are very deep indeed, and become separated from the endoderm by a thin structureless membrane (Text-fig. 35, *S. M.*). There is no direct evidence to show which layer secretes this, but it seems identical with and indeed an early part of, the meso-

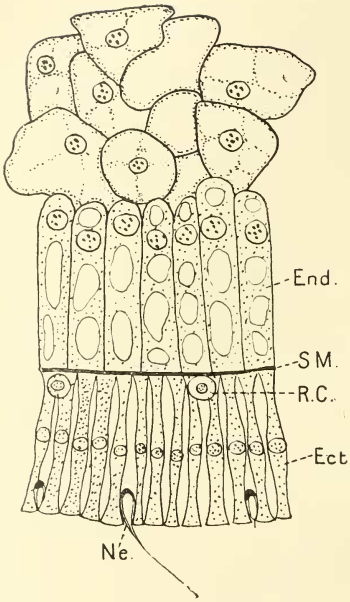
[1] Hickson (4a) describes the endoderm in the planula of Alcyonarians as a plasmodium, but from the present account it is clear that in Alcyonium digitatum the endoderm is always a definite cellular layer.

glœa of the adult polyp. This being so, the endoderm must be regarded as its source of origin (see p. 72 seq.).

The Planula.

On the fourth day the pre-planula merges into a spherical swimming planula of wavy outline (Text-fig. 4). Cilia are developed by the ectoderm cells, and a definite anterior pole is marked by being held foremost while swimming. The cœlenteron is still partly filled by groups and strings of loose endoderm cells, empty cells, protoplasmic cell contents and yolk. The definite endoderm consists of a row of columnar cells full of yolk vesicles, and roughly pyriform, i. e. rather wider at the free nuclear end than at the base. To the inner edge of the permanent endoderm one or more rows of rounded cells still adhere as in Text-fig. 36. The yolk has all been absorbed in the columnar ectoderm, the cell contents being now granular, while the separating membrane is much more distinct. These ectoderm cells have slightly expanded and flattened bases where they rest on the membrane, and are about as tall as the permanent endoderm cells, and half as wide. Rapid proliferation will again temporarily obscure the columnar character of this layer so that it seems to consist of several rows of round cells, but, as in the previous and subsequent stages, they soon return to their columnar state. In a few hours the round planula has become oval, but at first its outline remains sinuous. The microscopic structure is little altered, save that the degenerating endoderm cells have been absorbed in increasing numbers, while the permanent row of endoderm cells stand out clearly (Pl. 3, fig. 17, c., *End.*). By the fifth day the outline of the planula is smooth, and soon the anterior pole broadens, so that the larva assumes its characteristic pear-shape (Text-fig. 6). It rapidly lengthens, while the permanent row of endoderm cells still shows several rows of rounded or polygonal cells clinging to it in places, and often forming club-shaped projections into the cœlenteron (Pl. 3, fig. 1, and Pl. 3, fig. 17, *C. P.*). Increase in the number of endoderm

TEXT-FIGS. 35–37.



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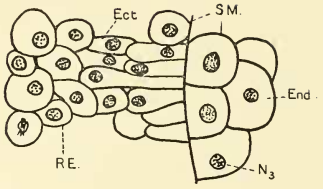

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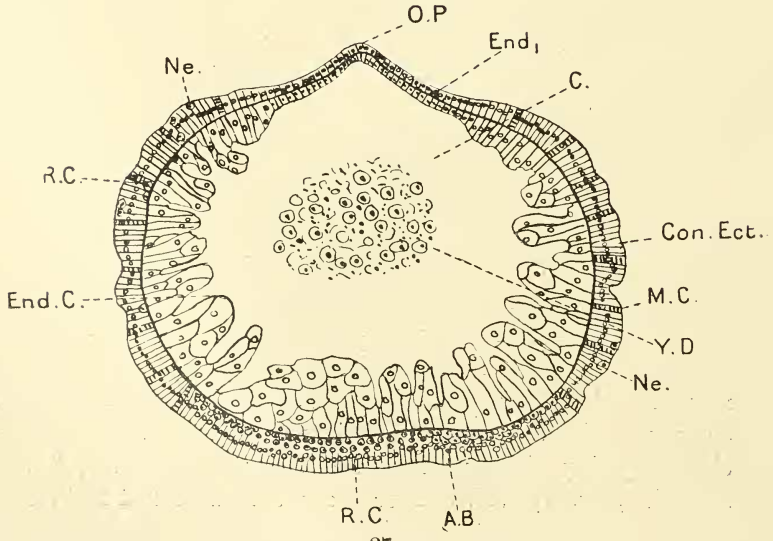

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and ectoderm cells is brought about by the multiplication of very small interstitial cells found at their bases. When very young these are even smaller than the nuclei of the adult cells. Nematocysts are now formed by the rounded cells lying at the outer edge of the ectoderm (Text-fig. 36), these cells being also recruited from the interstitial cells (Pl. 3, fig. 19, *Ne.*). Previous to this many ectodermic interstitial cells give rise to broad columnar mucous cells, i. e. refringent cells with a protoplasmic network surrounding the mucus which stains deeply with hæmatoxylin (Pl. 3, fig. 2, *M. C.*). At the anterior pole the endodermic tissue forms a deeper layer than elsewhere (Pl. 3, fig. 1). The ectoderm cells of the anterior pole become specially long in the well-grown planula, and the mucous cells are particularly numerous. Now the larva settles by the broad anterior and aboral pole (see general account), and for some hours after fixation the still ciliate planula hangs freely in the water, retaining its characteristic contractile power. If forcibly detached from the mucus plug which fastens the broad anterior end to the substratum, it will swim freely again for some time and then resettle. The round flat disc formed by the anterior pole becomes the base of the new polyp, and hence the nucleus of attachment of a fresh colony. For some time after settling the cœlenteron still contains much yolky detritus, which is gradually used up. The endoderm is similar to that described for the late planula, i. e. many cells deep at the fixed aboral pole and one layer elsewhere, clumps of non-permanent cells clinging to it in places (Pl. 4, fig. 24, *End. M.*, and *End.*). Very many round cells arise next to the supporting membrane in the ectoderm of the fixed base of the polyp, soon giving it a multilayered character (Pl. 4, fig. 23, *R. C.*). Mucous cells are still abundant in the ectoderm, but gradually disappear,

Fig. 35. Ectoderm of stage shown in Pl. 3, fig. 16, at time of rapid proliferation of cells (temporarily multilayered). Fig. 36. Ectoderm and endoderm cells from late planula, showing nematocysts. Fig. 37. Sagittal section of newly settled polyp, now shrinking in length. The pointed posterior and oral pole of the planula is still visible (× 133).

while nematocysts are very abundant over the whole surface. The long planula-shaped polyp now shrinks in length (Text-figs. 10, 11, and 37), the ectoderm and supporting lamella each becoming crumpled up on itself so that it is of wavy outline in section. The endoderm cells are indeed heaped up into a series of blunt processes separated by deep hollows, and projecting into the cœlenteron (Text-fig. 37, *End. C.*). Soon the pointed aboral end flattens down, the larva in vertical section now appearing like Pl. 4, fig. 23. The endoderm is a single row, except at the base, where it forms a deep multilayered mass of cells each containing one huge vacuole (in stained preparations). Thus a mound-like stage is reached at the end of the first day of sedentary life. A little later the mesenteries arise, and simultaneously a great and rapid increase in the number of rounded cells at the base of the columnar ectoderm occurs all over the surface. Next these become surrounded by mesoglœa, and from now onwards an intermediate layer separates the endoderm and ectoderm of the polyp. The rounded cells are young scleroblasts and nematocysts.

The next section of the paper deals with the secretion of mesoglœa, as the further development of the polyp may then be more easily understood.

9. Mesoglœa.

In this paper the word mesoglœa[1] will be applied to the structureless, deep-staining, jelly-like substance which lies between the ectoderm and endoderm of the polyp, and, aided by the spicules, gives rigidity to the body wall. The "endo-mesoglœal" cells are the cells which become embedded in it. The thin supporting lamella which first appears between the endoderm and the ectoderm of the late pre-planula, and can be traced in the planula (Pl. 4, fig. 27, *S. M.*) and earliest settled stages, stains quite similarly to the mesoglœa of later

[1] This word was introduced with the significance given above by Prof. Gilbert Bourne, F.R.S., in 1887 (see 'Quart. Journ. Micr. Sci.,' vol. 27, p. 303).

stages, and seems to be simply an earlier secreted part of it. At the time of the early formation of the mesenteries the lamella of the body wall is first thickened by further secretion of mesoglœa which is deposited on its outer side and separates it from the ectoderm. This process continues during later stages, and it is found that the most recently secreted part of the mesoglœa, i. e. that part lying nearest the ectoderm (Pl. 5, fig. 39, *R. Mes.*), always stains more feebly than the rest (*E. Mes.*). The mesoglœa, after thickening the supporting lamella of the attached base of the polyp (Pl. 5, fig. 40, *Mg.*), streams between the round cells of the multilayered ectoderm (*Ect.*) and unites with the original disc of adhesive mucus (*M. P.*), thereby strengthening the attachment of the polyp to the substratum. The mesoglœa is thickest in the body wall, much thinner in the bases of the tentacles, and very little thicker than the original supporting lamella in the distal ends of the tentacles, the pinnules, the oral disc, and stomodæum. No cells have been found embedded in the mesoglœa which could be shown responsible for its secretion. On the other hand, streams of newly secreted mesoglœa (Pl. 5, fig. 35, *S. Mg.*), which would seem in this stage to be a very viscous fluid, are found running outwards, firstly from the very slightly thickened supporting lamella (Pl. 5, fig. 35, *Mg.*), and later from the gradually thickening mesoglœa of the body wall (Pl. 5, fig. 36, *Mg.*) towards the ectoderm. They encroach on and finally surround the round cells at its base (Pl. 5, fig. 36, *Sc.* and *Ne.*), isolating them singly or in small groups in the mesoglœa (*S. cell* and *Gr. cell*). These mesoglœal cells arise as interstitial cells (Pl. 5, fig. 37, *Sc.*), and give rise either to spicules or nematocysts. The formation of the latter has not as yet been followed, but it was supposed during these investigations that the small rounded cells which stain very blue with picro-nigrosin and are only about half the size of the young scleroblasts when engulfed are young nematocysts. Perfectly formed nematocysts may also be thus surrounded, and occasionally a scleroblast secretes a young spicule while still lying at the base of the ectoderm, i. e.

before it becomes surrounded by mesoglœa. Evidence seems to indicate that the mesoglœa flows out from the endoderm (Pl. 5, figs. 36 and 39). It is certainly not secreted by any of the ectoderm cells, and the direction of flow is always outward from the endoderm to the ectoderm, and then, as previously stated, in among and around the basal cells of this layer. During the later growth of the mesenteries they consist almost wholly of a thin sheet of structureless mesoglea, covered on both sides by endoderm (Pl. 5, fig. 38, Mg_1 and *End.*), so that this layer is probably capable of providing for all further secretion of mesoglœa required by the growth of the mesenteries (see paragraph on mesenteries).

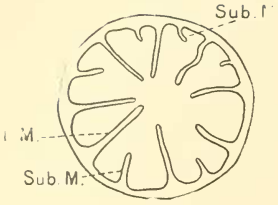
Nothing has been found to correspond with the irregular cells described by Bourne (**1**), full of minute highly refringent granules, which concealed the nucleus. He suggested that these might be the mesoglœa-secreting cells, while Woodland (**18**) described some small rounded cells which he also noticed as full of refringent granules, without deciding whether these corresponded to Bourne's. As both these papers were founded on work done on Alcyonium colonies, cells may be present in the mesoglœa which are not found in the solitary polyp. Still it seems very possible that Woodland's cells were merely young scleroblasts, judging from his figures. The "oval bodies" described by Hickson (**3**) and Woodland as occurring in the mesoglœa are nematocysts which may be in process of migration. The coiled thread in the cell was clearly stained with picro-nigrosin after preservation with osmic acid (Pl. 3, fig. 22, *N. T.*)

10. Mesenteries.

The eight characteristic mesenteries of Alcyonium are thin vertical sheets stretching radially into the cœlenteron from the body wall and dividing it into eight incomplete intermesenteric compartments, without meeting one another in the centre. They consist of thin sheets of mesoglœal substance arising from the supporting lamella of the body wall (Pl. 3, fig. 19, *Mes.*), and covered on both sides by endoderm cells

(*End.*), which in the early stages are long and columnar, but become low and broad later when the mouth has opened. The mesenteries arise simultaneously early on the second day of fixation, and usually do not appear until the flattening of the larva is complete. They are visible before the original supporting lamella of the body wall is thickened by the addition of fresh layers of mesoglœal substance, and therefore before the development of the spicules, tentacles, and mouth, these organs following them in the order stated. Between the normal eight as many as eighteen rudimentary mesenteries are often developed (Text-fig. 38, *Sub. M.*), but these either remain very small or eventually disappear, and are possibly vestiges of a more primitive condition when the mesenteries were very numerous (cf. Clavellina (**9**)). In colonial forms the new polyps formed by budding never show these rudiments. In the early stages the mesenteries are indicated from outside by eight equidistant shallow vertical grooves, stretching up a little way from the base of the lateral wall (Pl. 3, fig. 3), while sections of rather older stages indicate that the mesenteries are already arranged in four pairs (Text-fig. 39). At this stage the mesenteries in vertical radial section resemble Text-fig. 12, *c*. During the earliest stage observed (Text-fig. 40, *a*.) the supporting lamella of each mesentery, consisting of a very thin sheet of mesoglœa, can be easily traced. Sections show it growing radially inwards from the supporting lamella of the body wall (Text-fig. 41, *Mes.*) between the endoderm cells lining the latter. At this time it is structureless, stains slightly with picro-nigrosin, and encloses no cells. Each mesentery grows rapidly upwards along the body wall and along the base of the polyp towards the centre (Text-figs. 40, b_1, and 12, *c.* and *d.*). Meanwhile it increases in radial depth (Text-fig. 40, b_1), and as the supporting lamella deepens, the endoderm cells surrounding it grow inwards with it, so that each mesentery soon projects into the cœlenteron as a shallow ridge formed by an infolding of the endoderm supported by a central ridge, the lamella (Text-fig. 42, *Mes.*). Figures of subsequent stages show that the

TEXT-FIGS. 38–45.



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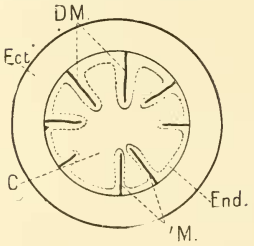

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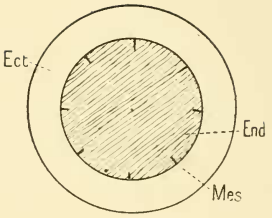

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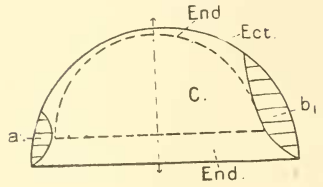

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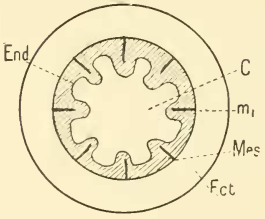

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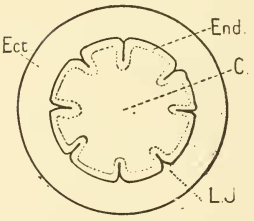

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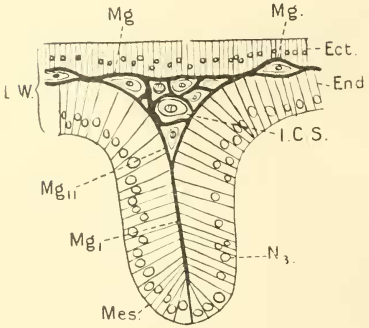

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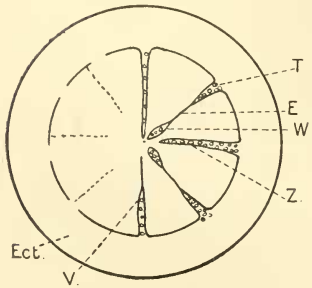

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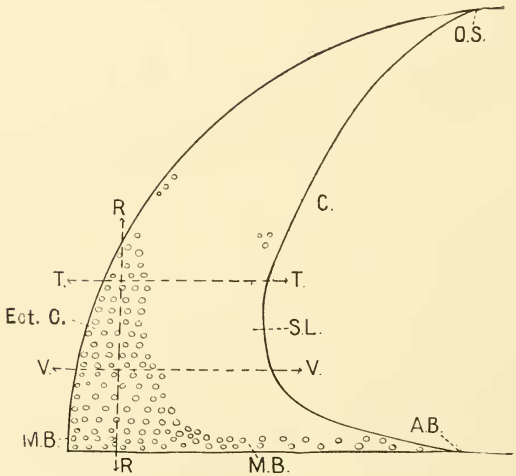


43A

mesentery is free along its inner edge but attached elsewhere to the body wall and base of the polyp. By the end of the second day the mesenteries nearly meet in the centre of the oral and aboral surfaces of the polyp, but they are still fairly shallow (Pl. 3, fig. 5, and Text-fig. 12, *d*.). After the formation of the tentacles the stomodæum develops as an invagination of the oral surface, in the space encircled by the upper edges of the mesenteries, and as it grows inwards the upper edges of the mesenteries are carried down with it, so that all eight become attached along the entire length of the stomodæum (Text-fig. 13, *f*. and *S*.). There has been some discussion as to whether a mesentery is purely endodermic in origin (the view taken by Wilson and Kowalevsky), or whether both the endoderm and ectoderm contribute to its formation (the view held by de Lacaze-Duthiers), and the following account may

Fig. 38. Aboral view of polyp, some hours older than Pl. 3, fig. 4. The eight permanent mesenteries are distinguished from the subsidiary ones by their greater development. Spicules just appearing (× 27). Fig. 39. Transverse section of polyp about stage drawn in Text-fig. 12, showing paired arrangement of mesenteries. Fig. 40. Vertical section of polyp with very young mesenteries, early on second day of fixation. The vertical dotted line divides the diagram into halves, the right showing a more advanced stage than the left. At *a*. a very young mesentery is drawn, which does not yet project beyond the endoderm. At b_1 a rather older mesentery is shown (at a stage similar to the mesenteries in Text-fig. 42). Fig. 41. Diagram of a transverse section of a young polyp near its base. The mesentery is similar to that shown in Text-fig. 40 (*a*). Hence section cuts through a solid layer of endoderm (the multilayered endoderm at the base of the young settled polyp), and through the supporting lamella of eight young mesenteries (*Mes*.). Fig. 42. Transverse section of a rather older polyp, at stage drawn in Text-fig. 40 (b_1), some distance higher up than the level of Text-fig. 41. The mesenteric ridges have deepened radially, and are covered by columnar endoderm cells. Fig. 43. Transverse section of polyp somewhat older than Text-fig. 42, showing how the lamella of the lateral wall becomes pulled in during the inward growth of the mesenteries. Fig. 43A. Lamella of young mesentery, reconstructed from sections. Fig. 44. Here the mesoglœa is being rapidly thickened. It is seen penetrating between the ectodermic cells which have been drawn into the root of the mesentery, and isolating them (*Mg*. and *I. C. S*.). Fig. 45. Transverse section of polyp, showing mesenteries cut across near base, i.e. at level *M. B*. in Text-fig. 43A.

serve to show how support for both views could be obtained in the case of Alcyonium according to the part of the mesentery studied :

(1) Sections show that by the time the mesenteries reach up to the oral surface of the polyp, the supporting lamella contains a great many ectoderm cells which lie very near the body wall. Text-fig. 43A is reconstructed from a series of sections of a polyp with mesenteries at this stage. It is a diagram of the supporting lamella of one mesentery, while the covering endoderm is not represented. Many small round cells are seen embedded in it towards the outer edge and at the base (*Ect. C.*). These are nematocysts and young scleroblasts which have entered from the ectoderm in a manner to be discussed later. Therefore both ectoderm and endoderm elements occur in the mesentery at this stage. However, it is seen that the greater part of the lamella contains *no* ectoderm cells, while as the mesentery grows larger the proportion of the lamella which contains ectoderm elements grow necessarily less and less, so that in the adult the mesentery is almost wholly endodermic in structure. This seems to support the view that the function of the ectoderm cells in the mesentery may be unimportant, and that the endodermic covering can provide for all further increase in the size of the lamella.[1]

(2) When the mesentery first develops, its supporting lamella grows radially inwards from the lamella of the body wall and is directly continuous with it (Text-figs. 41 and 42, *Mes.*). A little later, as the mesentery continues to grow inwards, the lamella of the lateral wall often appears slightly pulled in where the mesentery joins (Text-fig. 43, *L. J.*), and these two cases may appear in one section. Interstitial cells

[1] The fate of the nematocysts is uncertain: possibly they migrate to some definite place for future use. It is known that they occur in the six ventral mesenteric filaments, but probably all of these are derived from the ectoderm of the stomodæum, as described later. The spicules secreted by the scleroblasts found in the lamella would certainly help to stiffen it at the base and near the body wall.

are constantly arising in the ectoderm of the lateral wall, and some are often seen lying near the inner edge of the mesentery, and are therefore frequently found in the groove formed at this point. As the mesenteries grow these grooves deepen, and so more interstitial cells canenter. While the mesoglœa of the body wall is being laid down outside the original supporting lamella (Text-fig. 44, *Mg.*), it appears to flow between these interstitial cells, cutting them off from the ectoderm either singly or in small groups, just as it envelops and isolates interstitial cells in other parts of the body wall (see chapter on Mesoglœa). It is not impossible then that any ectoderm cells found in the mesentery are introduced by mechanical means, i.e. the ingrowing of the mesentery draws a few interstitial cells after it, which become enclosed in the supporting lamella, this process continuing in some regions until a large number are enclosed.

(3) Transverse sections of the mesentery in Text-fig. 43A at level *T.* (indicated by a dotted line) would show a few cells in the supporting lamella near the body wall. At level *V.* a similar condition would obtain. Lower still, the lamella in cross-section would show a very deep groove full of cells near the lateral wall, and a very short single sheet or none at all (Text-fig. 45, *V.* or *Z.*). Towards the base of the mesentery the lamella is double and full of ectoderm cells, or in some cases it contains a group of cells near its inner edge (Text-fig. 45, *W.*), cut off from the outer deep groove *T.* by a region *E.*, containing no cells. A vertical section of Text-fig. 43, *A.*, at line *R.* would obviously show a double lamella full of ectoderm cells and surrounded by endoderm, and this would appear to be the kind of section figured by de Lacaze-Duthiers (**10**).

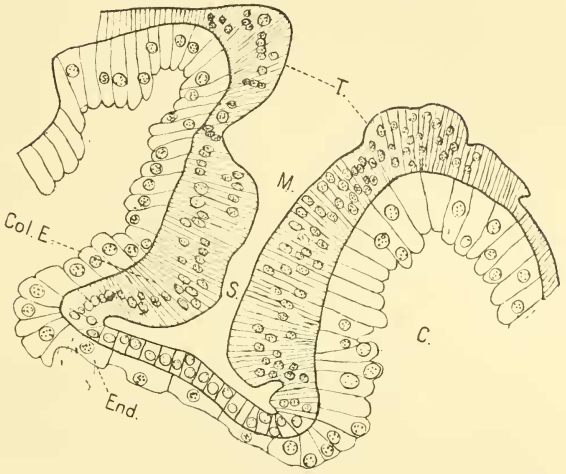
Considered apart from the early history of the mesentery, Text-fig. 45 seems to indicate that the mesenteries are true invaginations of the body wall. A solid cord of ectoderm cells seems to grow in, surrounded by a tubular ingrowth of the supporting lamella, and a covering of endoderm cells, but whether the former cells are to be regarded as drawn in

mechanically during the ingrowth of the mesentery, or whether they have any embryological significance implying a true invagination of both layers is not altogether certain. If the first, then the mesentery is purely endodermic, as Wilson believes; if the second, then de Lacaze-Duthiers is correct. The total evidence afforded by these investigations seems, perhaps, to support the latter view. It may be added that the rudimentary mesenteries contain many ectoderm cells embedded in the supporting lamella.

11. Tentacles, Mouth and Stomodæum.

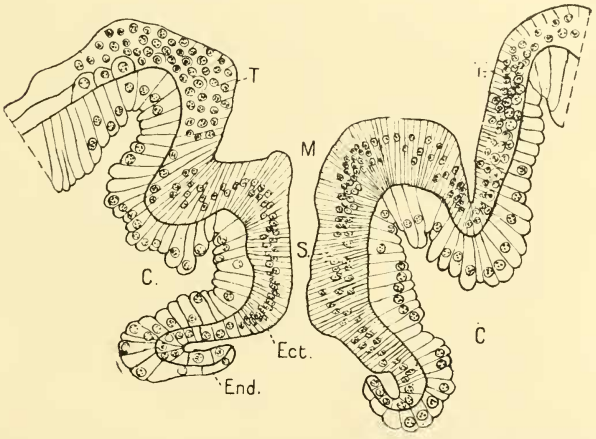
Eight short broad tentacles develop on the third day (Pl. 3, fig. 5). They are simple hollow outgrowths of the upper surface of the young polyp, consisting, therefore, of ectoderm and endoderm separated by a supporting lamella (Text-figs. 46 and 47, *T.*), and arise between the mesenteries in a circle round the oral disc. A little later a shallow invagination of the latter forms the beginning of the stomodæum. This grows considerably in size and depth, the exterior and only opening being the mouth (Pl. 3, fig. 7, and Pl. 4, fig. 28). The lumen of the invagination soon widens considerably at the base, and the floor of the cavity is correspondingly enlarged (Text-fig. 46). During the fourth day the endoderm and ectoderm of this floor degenerate, either at the centre or near the side or in both regions simultaneously (Text-fig. 47). By this means communication is established between the cœlenteron and the exterior, and food may enter from outside. Until now all requisite nourishment has been provided by the embryonic yolk. By the fifth day the disintegration is complete, and the formation of the mouth and stomodæum is accomplished. The ectoderm covering the latter consists of taller columnar cells than elsewhere. Like the tentacles it is plentifully supplied with nematocysts, which, indeed, at this stage are abundant all over the surface of the polyp. Mucous cells and granular gland cells are soon formed in the stomodæum and pour their

TEXT-FIG. 46.



Longitudinal section of the stomodæal invagination, just before floor of canal begins to degenerate (× 380).

TEXT-FIG. 47.



Same, when communication is established between the cœlenteron and the exterior, by the degeneration of the endoderm and ectoderm at the centre of the base.

secretion upon the food while it is being passed down. The supporting lamella of the stomodæum always remains a thin sheet, while the endodermic lining is similar to that found throughout the cœlenteric cavity, as the endoderm covering the attached base of the polyp is by now reduced to one row of columnar cells (Pl. 3, fig. 19, *End.*). In section the top of the newly-opened stomodæum is round, but lower down it is keyhole shaped (Pl. 4, fig. 34), the narrow ventral end of the keyhole being the siphonoglyph. Hence in vertical sections which cut the stomodæum dorso-ventrally, the latter seems much wider than when cut in a plane at right angles to this.

12. Spicules.

Investigation into the origin of the spicule in the young solitary polyp confirms Woodland's work on colonial forms (18), adding thereto one or two minor points of interest. Each spicule, as he states, is the product of a single cell, and during its elaboration the nucleus halves, each daughter nucleus apparently controlling one pole of the spicule (Pl. 4, fig. 31, Nos. 1 and 5). It was found, however, during the present investigations that some large spiculoblasts from Alcyonium colonies contained three or four nuclei. It was also seen that the young scleroblasts are of ectodermic origin (Pl. 4, fig. 32), and arise as round interstitial cells at the base of the ectoderm of the body wall and tentacles (Pl. 5, fig. 35, *Sc.*). These scleroblasts become stellate, spindle-shaped, oval or rounded, as they increase in size (Pl. 4, fig. 32), and, as explained previously, are encroached on and eventually surrounded by mesoglea, either singly or in small groups. In later stages the spicules become entirely isolated from one another by mesoglœa. As Woodland states, the cytoplasm of the scleroblasts eventually becomes reduced to a mere thin granular covering-layer over the greatly enlarged spicules (Pl. 4, fig. 31, Nos. 4 and 5). Pl. 4, fig. 30, shows that after dissolving away the spicules by an acid stain (picro-nigrosin), the mesoglœa is left full of corresponding

cavities lined with the cytoplasm of the scleroblast, each cavity shaped exactly like the spicule which occupied it. The earliest spicules arise on the second day of fixation, rather later than the mesenteries and before the tentacles, and may be examined with low powers of the microscope. They appear as small refringent nodules in the upper surface of the mound-shaped polyp, and show through by transmitted light. One row of small unbranched spicules is present in the body wall by the time the mouth opens (Pl. 5, figs. 36 and 37), and in older polyps they are found in the upper and lower regions of the body wall, the bases of the tentacles, and in the outer edge of the mesenteries very near the base (Text-fig. 14). According to Woodland, after the two daughter nuclei have formed in the scleroblast, the steadily growing round spicule lengthens and assumes a simple dumb-bell shape (Pl. 4, fig. 31, No. 3). The ends of this dumb-bell become gradually elaborated into processes, so that the spicule then resembles a caudal vertebra in shape (Nos. 4 and 5). The colonies he utilised in following out the development of the spicule were small—about half an inch across—and the drawings he made have all been verified on the solitary polyp, with the exception of his figures illustrating the cavities which the spicules occupied.

13. Mesenteric Filaments.

Each mesentery bears a filament on its free inner edge running down for some distance from the lower opening of the stomodæum (Text-fig. 48). While the two dorsal filaments create an upward current of water in the cœlenteron by the active lashing of the cilia borne on the cells which cover them (Text-fig. 49), the six ventral filaments are secretory and absorptive. The ferment poured upon the food by these filaments continues and probably completes the disintegration begun in the stomodæum. In the oldest solitary polyp examined (fixed from thirteen to fifteen days), the ventral filaments reached almost to the bottom of the free

edge of their respective mesenteries, and the dorsal not quite so far (Text-fig. 48). The first signs of each ventral filament

TEXT-FIGS. 48–51.

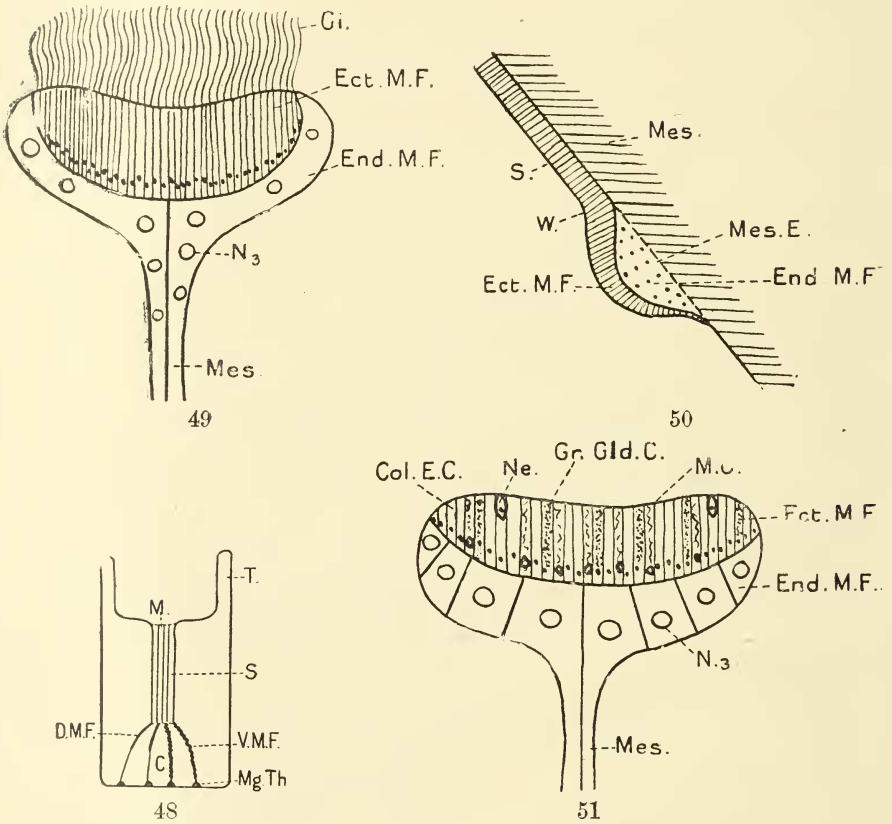


Fig. 48. Vertical section of polyp on thirteenth day of fixation, showing relative length of dorsal and ventral mesenteric filaments. Fig. 49. Transverse section of young dorsal mesenteric filament. Fig. 50. Longitudinal and radial section of very young ventral mesenteric filament, showing its relation to the thickened edge of the mesentery. Fig. 51. Transverse section of young ventral mesenteric filament.

are found on the fourth day of sedentary life, shortly before the mouth is open. Immediately below the base of the

stomodœal invagination the free edge of the mesentery becomes thickened by a proliferation of the endoderm cells in that region. These cells are longer than they are wide, whereas those on the rest of the mesentery are short and broad. After the mouth and stomodæum have completed their formation, on the fifth day, a narrow strap-like process of the ectoderm of the stomodæum grows out over the endodermic part of each filament (Text-fig. 50, *Ect. M. F.*, and Pl. 4, fig. 33, *E. S. O.*; cf. Wilson's fig. 21 (**15**)). In this ectodermic band, which is only 6 μ (or one section) broad at first, nematocysts, mucous and granular gland cells develop. The band, lying over the endodermic part of the filament, soon widens and forms an ectodermic layer covering the outer surface of the latter (Text-fig. 51). [In this figure the two layers in the filament are marked *Ect. M. F.* and *End. M. F.*, and are shown in transverse section, while Text-fig. 50 represents a much earlier stage in longitudinal section (about stage of Pl. 4, fig. 33), the dotted line marking the edge of the mesentery before the endodermic thickening began (*Mes. E.*). The letter *W.* marks the lower limit of the stomodæal wall (*S.*), before the ectodermic downgrowth developed.] By the sixth day these filaments extend half way down the free edge of the mesentery (Pl. 4, fig. 25, *S. O.*). During subsequent growth the filament becomes somewhat twisted laterally and gathered up into puckers on the edge of the mesentery as a result of growing faster than the latter. Consequently, sometimes the ectoderm and sometimes the endoderm cells lie uppermost, and this can be realised easily by imagining the filament shown in transverse section in Text-fig. 51 twisted from side to side. Longitudinal sections through the convoluted filaments are therefore somewhat difficult to understand at first sight, as groups of ectoderm and endoderm cells lie side by side.[1]

While the ectodermic portion is secretive, the endodermic

[1] A model in red and white clay of the endodermic and ectodermic parts of the filament was similarly twisted from side to side and then sectioned to check this result.

part is absorptive. Food is engulfed by the amœboid processes of the latter cells in common with the endoderm cells lining the general body cavity (12). The above observations probably explain why in Miss Pratt's paper ((12) Pl. 21, fig. 4) the carmined food is absorbed, and has reddened the filament in certain definite areas and not in others. The colourless parts are the ectodermic and the reddened parts the endodermic areas twisted uppermost. It also explains the observation made early in the same paper, that histological study of the "stomodæum and ventral mesenterial filaments in several members of the family reveals many points of similarity, if not identity, in their elemental constitution. Both granular and mucous gland cells, as well as nematocysts, occur in all these structures." Briefly, this is because the secretory part of these filaments *is* ectodermic, the requisite gland cells and nematocysts being supplied by the downgrowth from the stomodæum. The dorsal filaments are very much narrower and straighter than the ventral in the solitary polyp. A transverse section of these dorsal filaments two days after they first appear is only two-thirds the size of a similar section of the ventral filaments on the same date. No indication of these dorsal filaments is found until the sixth day, i.e. they arise later than the others. Narrow processes of the stomodæal ectoderm are then seen growing down over the uppermost part of the free edge of the two dorsal mesenteries, thus giving rise to the filaments (Pl. 4, fig. 26, *D. O.*). No appreciable thickening of the endoderm forms, however, as a support for this ectoderm, whereas it was visible before the ectodermic part in the ventral filaments. A transverse section reveals that in rough outline the dorsal filament is quite comparable to the ventral, the difference being one of degree of development only (Text-figs. 49 and 51). In the former the ectodermic band, consisting of tall ciliate cells with deep staining nuclei, rests on a slender endodermic support, which is smaller than in the ventral filament. Therefore both kinds of filament consist of ectodermic and

endodermic portions, the endodermic being well developed in the ventral, and much less so in the dorsal. Growth seems slower in the dorsal than the ventral filaments, so that by the end of the seventh day they are still much shorter than the latter (Pl. 4, fig. 26).

Sections made of polyps for the examination of the filaments at this stage, also show that the retractor muscles of the mesenteries are now developing. By the thirteenth to fifteenth day of sedentary life the ventral filaments are twice as long as the dorsal, although they extend very little below them, because of their convoluted condition. By this date, also, the filaments approximate more nearly to the adult in transverse section, i.e. the ciliated surface of the dorsal filament has become more concave, while the glandular ectoderm of the ventral has become more convex, and extends further round (cf. Text-figs. 49 and 51 with Pl. 4, fig. 29, and (3) Pl. 38, figs. 18 and 19).

Summary of other Writers' Views on the Derivation of the Mesenteric Filaments, and Remarks on these.

H. V. Wilson (**15**) considers that the ventral mesenteric filaments in the coral Manicina are wholly ectodermic in origin, and gives a very similar figure to the present Pl. 4, fig. 33, showing the downgrowth from the stomodæum of the ectodermic bands which give rise to the filaments. E. B. Wilson (**17**) states that the dorsal filaments in colonial polyps of Alcyonium are of ectodermic origin, but while he shows (**16**) that endoderm certainly enters into the ventral filaments of Renilla, he did not find any ectodermic outgrowth contributing to them. It is not impossible that he passed over this stage in development. J. Stanley-Gardiner (**13**) considers that the ventral mesenteric filaments of Cœnopsammia are purely ectodermic, basing this view on histological grounds. Also he mentions the same fact for Flabellum (**14**).

It is possible that investigations into the early development of other Anthozoa would confirm the fact that all the filaments throughout the group consist of ectodermic and endo-

dermic elements, both being well developed in the ventral, while the ectodermic part is alone elaborated in the dorsal.

14. Summary.

(1) The fertilised eggs segment in various ways, but typical morulæ always result.

(2) When the sixteen cell stage again divides to produce the thirty-two celled embryo, delamination occurs, and from now onwards the larva is two-layered.

(3) The morula at the twentieth hour begins to undergo a series of contortions which last from the first to the third day. This solid contorted stage is here termed the pre-planula, as it passes on into the hollow planula stage.

(4) The pear-shaped planula, while swimming, exhibits characteristic "planarian-like" movements, and on the fourth free-swimming day (the seventh day of development) it settles down by the broad anterior and aboral pole.

(5) The settled larva soon flattens, assuming a mound-like shape, and on the second day of fixation eight mesenteries grow out simultaneously into the cœlenteron from the base of the lateral wall. The cœlenteron is identical with the hollow central space in the endoderm of the planula.

(6) The mesenteries, arranged in four distinct pairs, grow simultaneously and rapidly along the lateral walls and attached base, and soon nearly meet on the basal and oral surfaces of the polyp.

(7) Many round cells now appear at the base of the columnar ectoderm.

(8) Mesoglœa is at this time secreted by the endoderm, and flows round the above-mentioned round ectoderm cells, cutting them off either singly or in groups. These isolated ectoderm cells produce either nematocysts or spicules, the spicules appearing soon after the mesenteries.

(9) Early on the third day eight simple hollow tentacles grow out, alternating with the mesenteries, and encircling the oral surface.

(10) Later on the third day the stomodæum and mouth arise by the appearance of a rapidly deepening invagination of the oral surface, in the centre of the circle of tentacles. Yolky detritus is still present in the cœlenteron.

(11) On the fourth day the base of this invagination degenerates, and so the cœlenteron communicates with the exterior.

(12) While the mouth invagination is still in process of formation, the endodermic portion of the mesenteric filaments arises on the six ventral mesenteries by a proliferation of cells on the upper part of the free edge.

(13) On the fifth day the ventral mesenteric filaments are completed by strap-like ectodermic downgrowths from the stomodæum over the endodermic thickening on each mesentery.

(14) On the sixth day the dorsal mesenteric filaments arise.

(15) The dorsal and ventral mesenteric filaments appear homogeneous in origin, though of diverse function.

(16) On the seventh day the eight filaments reach about half way down the free edges of their respective mesenteries.

(17) Further development consists of elaboration of the organs already present.

(18) At the end of the third week the first bud is formed, and the solitary polyp becomes a young colony by stolonal gemmation.

(19) The young colonies were successfully fed in the laboratory on larvæ and adult individuals from colonies of Leptoclinum and Botryllus.

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EXPLANATION OF PLATES 3–5,

Illustrating Mrs. Annie Matthews's paper on "The Development of Alcyonium digitatum, with some notes on the Early Colony Formation."

Reference Letters of Plate Figures.

a. Radial vertical section of very young mesentery, before it projects into the cœlenteron. a_1. First developed bud of colony. *A. B.* Fixed base of polyp. *An.* Anterior aboral pole. *b*. Small blastomere. b_1. Radial vertical section of mesentery, rather older than stage (*a*.) of same text-fig., and stretching higher up lateral wall of polyp. b_{11}. Second bud. *B.* Large blastomere. *B. Sp.* Spicules of body wall. *c*. Mesentery: somewhat older than stage (b_1.) of Text-fig. 40. c_1. Third bud. *C.* Cœlenteron. *C. End.* Columnar endoderm. *C. M.* Undivided central part of egg. *C. P.* Club-shaped processes of endoderm. *Col. E.* Columnar ectoderm. *Col. E. C.* Columnar ectoderm cell. *Con. Ect.* Contracted and wrinkled ectoderm. *Ci.* Cilia. *d*. Mesentery at a stage rather older than (*c*.) of the same figure. *d. Ect.* Deep columnar ectoderm in stomodæum. *d. m.* Dorsal mesenteries. *D. A.* Deeply staining, finely granular area round nucleus. *D. M.* Dorsal mesentery. *D. M. F.* Dorsal mesenteric filament. *D. N.* Delamination spindle. *D. O.* Dorsal filament just arising as downgrowth of ectoderm of stomodæum over free edge of mesentery. *E. Mes.* Earlier secreted mesoglea. *E. S. O.* Early stage of formation of ventral mesenteric filament, showing ectodermic band growing from stomodæum over edge of mesentery (*Mes.*). *Ect.* Ectoderm. *Ect. M. F.* Ectodermic part of mesenteric filament. *Ect. d.* Deep columnar ectoderm at base of lateral wall. *Ect. C.* Ectoderm cells. *End.* Endoderm. End_1. Endoderm cells, one row deep in this region. *End. C.* Contracted endoderm. *End. M.* Endoderm, several rows deep, lining attached base of polyp. *End. M. F.* Endodermic part of mesenteric filament. *End. S.* Endoderm cells in region where they are several rows deep. *f*. Radial vertical section of mesentery, at time when stomodæal invagination is forming. *F.* First row of buds. *G.* Glass. *G. P.* Granular protoplasm. *Gr.* Granular, yolk free area. *Gr. cell* Group of cells becoming embedded in mesoglœa. *Gr. E.* Granular ectoderm. *Gr. Gld. C.* Granular gland cell. *I. C.* Interstitial cells. *I. C. S.*

Interstitial cells cut off singly from octoderm by mesoglœa. *l.* Small lobe. *L.* Large lobe. *L. J.* Junction of supporting lamella of mesentery with that of body wall. $L_1 W_1$. Somewhat contracted and therefore wrinkled lateral wall of polyp. *L. W.* Lateral wall of polyp. m_1. Supporting lamella of mesentery growing radially inward from lamella of body wall. *M.* Mouth. *M. B.* Base of mesentery. *M. C.* Mucous cell. *M. F.* Mesenteric filament. *M. P.* Adhesive plug of white mucus. attaching base of polyp to substratum. *Mes.* Mesentery. Mes_3, Mes_5, Two lateral mesenteries. *Mes. E.* Edge of mesentery before ventral filament developed. *Mg.* Mesoglœa. Mg_1. Mesoglœa of mesentery. Mg_{11}. Mesoglœa flowing between interstitial ectoderm cells at root of mesentery. *Mg. Th.* Thickened ridge of mesoglœa stiffening the line of attachment of mesentery to base of polyp. *N.* Nucleus halved by karyokinesis. N_2. One of daughter nuclei, formed before first segmentation. N_3. Nucleus. *N. T.* Nematocyst thread. *Ne.* Nematocyst. Ne_1. Nematocyst, in mesoglœa of mesentery. Ne_4. Nematocyst, with thread stained. *O. P.* Oral pole. *O. S.* Oral surface, surrounded by tentacles. *P.* Pinnule. P_1. Earliest developed pinnule on the tentacle. P_2. Latest and youngest pinnule on the tentacle. *P. C.* Pre-oral cavity. *P. M.* One of eight permanent mesenteries. *Pa.* Parent polyp. *Pl.* Remains of degenerate base of stomodæal invagination. *R. C.* Rounded cells in ectoderm. *R. E.* Rapidly proliferating and therefore temporarily multilayered ectoderm. *R. Mes.* Recently secreted mesoglœa. *R. Sc.* Remains of scleroblast. *S.* Stomodæum. *Sc.* Scleroblast. *Se.* Second row of buds. *Su.* Substratum. *St.* Stolon. *Sp.* Spicule. Sp_1. Spicule embedded in mesoglœa. *Spb.* Spiculoblast with spicule dissolved out. Spb_1. Spicule left in scleroblast, embedded in mesoglœa. *S. cell.* Cell embedded singly in mesoglœa. *Sub. M.* Subsidiary mesentery. *S. B.* Termination of wall of stomodæum. *S. C.* Segmentation cavity. *S. L.* Supporting lamella. *S. M.* Separating membrane. S_1 M_1. Position occupied by supporting membrane in mesoglœa of body wall. *S. Mg.* Mesoglœa streaming out from endoderm among rounded cells at base of ectoderm. *S. O. S.* Sloping oral surface. *S. O.* Early stage in appearance of strap-like outgrowth of ectoderm of stomodæum, over endodermic part of ventral mesenteric filament. *T.* Tentacle. *T. B.* Swollen base of tentacle. T_1 B_1. Very young bud of the third row. *T. R.* Tentacles retracted. *T. Sp.* Tentacular spicules. *V. M.* Ventral mesentery. *V. M. F.* One of the six ventral mesenteric filaments. *V. M.* F_1. Lower limit of ventral mesenteric filament. *Wr.* Wrinkled outline of latest pre-planula stage. *Y.* Yolk globules. Y_1. Small yolk globules. Y_2. Large yolk globules. *Y. D.* Yolky detritus. *Y. E.* Inner half of columnar ectoderm cells, still containing yolk globules. *Y. V.* Large yolk-containing vacuoles.

PLATE 3.

Fig. 1.—Sagittal section of long highly contractile planula, towards end of free-swimming life. Yolky detritus still present in cœlenteron. Ectoderm very full of mucous cells and nematocysts. × 78.

Fig. 2.—Portion of ectoderm of full-grown planula, showing mucous cells and nematocysts among the ordinary cells. Granular reticulate protoplasm in mucous cells very deeply stained. × 507.

Fig. 3.—Lateral view of polyp with older mesenteries than fig. 4. Second day of fixation. Adhesive plug shows well. × 16.

Fig. 4.—Lateral view of polyp during early part of second day of attachment. Eight mesenteries appearing. × 16.

Fig. 5.—Lateral view of polyp on the fifth day of attachment. Tentacles partly contracted, body expanded. × 37.

Fig. 6.—Oral view of young polyp, with tentacles well expanded and mouth widely opened. The tentacles were still reddish-yellow and the body wall cream; spicules abundant (about five days fixed). × 35.

Fig. 7.—Oral view of polyp about the sixth day. Tentacles partly contracted, but mouth still exposed. × 39.

Fig. 8.—Section through the eight-lobed egg of Text-fig. 30. × 78.

Fig. 9.—Sagittal section through eight cell stage. One of the several nuclei that have again divided ready for the next segmentation is shown at *N*. × 78.

Fig. 10.—Section through an egg protruding sixteen lobes; two daughter nuclei are drawn. × 78.

Fig. 11.—Sagittal section through sixteen cell stage, showing delamination spindle. × 78.

Fig. 12.—Transverse section of a young morula showing delamination and ordinary spindles. × 78.

Fig. 13.—Enlarged drawing of section of two blastomeres from the sixteen cell stage. The finely granular outer area, and large and small yolk globules are shown. × 133.

Fig. 14.—Five endoderm cells from a late pre-planula, in which the lobes are almost withdrawn again. The yolk globules are few and very large and the segmentation cavity has disappeared. × 760.

Fig. 15.—Ectoderm and endoderm cells from transverse section of a late pre-planula, when the ectoderm cells have become much longer nd narrower, while large vacuoles are still present. × 78.

Fig. 16. Transverse section of a very late pre-planula, with rapidly proliferating ectoderm (stage of Text-fig. 3), just before it passes into

the early round planula stage. A definite membrane is now present between the ectoderm and the endoderm. × 78.

Fig. 17.—Sagittal section of smooth oval planula, with broadened anterior end. Endoderm cells in places only one row deep, but in others form club-like processes. (Second free-swimming day.) × 133.

Fig. 18.—Ectoderm and endoderm cells from a somewhat younger pre-planula than Pl. 3, fig. 14, and at a later stage than Text-fig. 2. Yolk globules fewer than in early pre-planula and increasing in size. × 760.

Fig. 19.—Part of a vertical section of a young polyp with mesenteries and spicules. One mesentery is cut through vertically near its attachment to the lateral wall. Many scleroblasts and nematocysts are thus cut across, which have been drawn into the mesoglœa of the mesentery here. At the lower right-hand corner young scleroblasts and nematocyst cells are shown in situ at the base of the columnar ectoderm. × 380.

Fig. 20.—Ectoderm and endoderm cells from a late morula stage. × 133.

Fig. 21.—Transverse section of an earlier morula stage than fig. 20, showing large yolk globules in the endoderm and smaller ones in the cuboid ectoderm. × 167.

Fig. 22.—Nematocysts (oval bodies of Hickson), from mesoglœa of solitary and colonial polyps.

PLATE 4.

Fig. 23.—Sagittal section of a polyp about stage shown in fig. 3. This section does not cut through the mesenteries. × 133.

Fig. 24.—Sagittal section of newly-fixed larva, still planula-shaped and ciliate. At the base the outer edge of the ectoderm has been diagrammatically thickened in the drawing, to indicate clearly the extent of the fixed area. × 133.

Fig. 25.—Vertical section of polyp with only one pinnule on tentacles, i.e. about six days fixed. At Mes_2 and Mes_4 the basal attachments of the two lateral mesenteries Mes_3 and Mes_5 are seen. At *V. M.* F_1 is shown the termination of the ectodermic outgrowth forming the mesenteric filament of mesentery Mes_3, i.e. about half way down the free edge of the mesentery. At *S. O.* the continuity of the stomodæum and this outgrowth is shown. × 133.

Fig. 26.—Vertical section of a polyp fixed from six to seven days, showing the strap-like ectodermic process on the right, which forms the ventral mesenteric filament, and on the left the shorter ectodermic

outgrowth over the free edge of the dorsal mesentery, which forms the dorsal mesenteric filament, *D. O.* The dorsal mesentery is only partly shown at *D. M.* × 133.

Fig. 27.—Part of ectoderm and endoderm of swimming planula. Multilayered and adjacent one-layered endoderm both shown. Granular ectoderm cells expanded at base rest on the separating membrane. Round interstitial ectoderm cells seen. Endoderm still full of yolk globules. × 760. (Ectoderm and endoderm of the pre-planula in the latest stage are very similar in detail.)

Fig. 28.—Vertical section of polyp showing stomodæal invagination. There is as yet no communication with the exterior. Yolky detritus is still present, and mesoglea is flowing between the ectoderm cells of the attached base, and round the inner ectoderm cells of the lateral walls. × 133. (The stomodæal invagination is cut across laterally, and so is very narrow.)

Fig. 29.—Transverse section of polyp settled from thirteen to fifteen days, cut below stomodæum; the six ventral and two dorsal mesenteric filaments are shown. × 133.

Fig. 30.—Section through the mesoglœa of a fairly old polyp after dissolving the calcareous part of the spicules by staining with picro-nigrosin. The nuclei and organic remains of the spicules are shown, and it can be seen that the cavities occupied in the mesoglea by the spicules are replicas of the latter. × 245.

Fig. 31.—Young spicules in scleroblasts, from solitary polyp. × 760.

Fig. 32.—Young scleroblasts before secretion of spicule. × 760.

Fig. 33.—Vertical section through the stomodæum of a polyp five days fixed. The remains of the degenerate base of the stomodæum are still visible, and the ectoderm of the stomodæum is growing down over a mesentery as a strap-like process (*E. S. O.*); the section is beyond the actual mouth opening. The rest of the mesentery (*Mes.*) was seen in subsequent sections. × 450.

Fig. 34.—Transverse section of a polyp settled from thirteen to fifteen days, cutting through the siphonoglyph. × 133.

PLATE 5.

Figs. 35, 36, 37 and 39.—Vertical radial sections of part of wall of young settled polyp, showing ectoderm, endoderm and origin of mesoglœa:

Fig. 35.—Mesoglœa secreted by endoderm, beginning to stream between the interstitial cells at the base of the ectoderm, at the time of the early formation of the mesenteries. × 380.

Fig. 36.—Streams of mesoglœa flowing between ectodermic interstitial cells, and cutting them off singly (*Spb.*), or in groups (*Gr. cell*). × 760.

Fig. 37.—Scleroblasts surrounded by mesoglœa. (The spicules have been dissolved during preservation.) The mesoglœa is seen streaming round the interstitial cells at the left hand of the diagram. × 760.

Fig. 38.—Part of a transverse section of a young polyp, cutting through stomodæum and mesenteries, and showing the thin sheets of mesoglœa, devoid of cells which together with the surrounding endoderm form the mesenteries. × 380.

Fig. 39.—Faintly staining streams of mesoglœa, flowing in between the newly formed interstitial cells, from the endoderm. × 760.

Fig. 40.—Vertical section of part of attached base of young polyp. The mesoglœa is shown streaming in between the ectoderm cells, and then strengthening the mucous plug. × 608.